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Toxicity assessment of 4-chlorophenol to aerobic granular sludge and its interaction with extracellular polymeric substances

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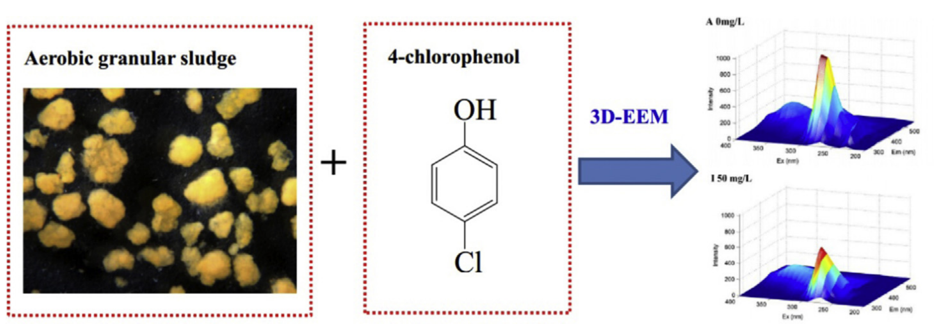
HIGHLIGHTS

- Toxicity of 4-CP to aerobic granular sludge process was evaluated.
- 3D-EEM characterized the interaction between EPS and 4-CP.
- Tryptophan was the main substance result in fluorescence quenching.
- The mechanism of fluorescence quenching belongs to static quenching.

GRAPHICAL ABSTRACT



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ABSTRACT

The main objective of this study was to evaluate the toxicity of 4-chlorophenol (4-CP) to aerobic granular sludge in the process of treating ammonia rich wastewater. In the short-term exposure of 4-CP of 5 and 10 mg/L, ammonia nitrogen removal efficiencies in the batch reactors decreased to 87.18 ± 2.81 and $41.16 \pm 3.55\%$, which were remarkably lower than that of control experiment ($99.83 \pm 0.54\%$). Correspondingly, the respirometric activities of heterotrophic and autotrophic bacteria of aerobic granular sludge were significantly inhibited in the presence of 4-CP. Moreover, the main components of extracellular polymeric substances (EPS) including polysaccharides and proteins increased from 18.74 ± 0.29 and 22.57 ± 0.34 mg/g SS to 27.79 ± 0.51 and 24.69 ± 0.38 mg/g SS, respectively, indicating that the presence of 4-CP played an important role on the EPS production. Three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectroscopy further showed that the intensities of EPS samples were obviously quenched with the increased of 4-CP concentrations. To be more detailed, synchronous fluorescence spectra indicated that the interaction between EPS and 4-CP was mainly caused by tryptophan residues. The mechanism of fluorescence quenching belongs to static quenching with a formation constant (K_A) of 0.07×10^4 L/mol, implying the strong formation of EPS and 4-CP complex. The results could provide reliable and accurate information to determine the potential toxicity of 4-CP on the performance of aerobic granular sludge system.

1. Introduction

With the development of the production and application of toxic chemical compounds, it is found that the toxic compounds in the industrial wastewater have a negative impact on

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wastewater treatment plant. Recently, many researchers have focused on the influence of toxicity substrate on biological nitrogen removal. Ricco et al. [1] evaluated the toxicity of four xenobiotic compounds, including 3,5-dichlorophenol, formaldehyde, 4-nitrophenol and dichloromethane in industrial wastewater contaminants by using a simple respirometric procedure. Kim et al. [2] found that the nitrification process was occasionally upset by serious inhibitory effects of toxic compounds (phenols, cyanides, and thiocyanate) in cokes wastewater.

However, most of the literatures related to the toxic investigation were assessed by using conventional activated sludge system. Aerobic granular sludge, as a novel biotechnology, has attracted intensive attention owing to its excellent physical characteristics [3]. Compared with activated sludge, aerobic granular sludge has a higher biomass concentration, a denser and stronger microbial aggregate structure, and more excellent settling capacity. Aerobic granules have a great potential for the treatment of various wastewaters, including high strength wastewater containing organics, nitrogen, phosphorus and heavy metals [4]. Moreover, the granule could be divided into three zones, i.e. an aerobic zone followed by a micro-oxygen zone and an anoxic (or anaerobic) zone with a wide range of micro-environments through the dissolved oxygen (DO) gradient [5]. Therefore, the response of aerobic granular sludge to toxic compounds may be different with that of activated sludge because of its unique granule attributes. However, little information is available regarding to this point by now, especially in relation to nitrogen rich wastewater because toxic organics are largely used in the industrial production process.

In particular, it has been proved that extracellular polymeric substances (EPS) are usually the first barrier of microbial cells that directly contact and interact with the toxic substance [6]. EPS are one of the complex high-molecular-weight mixtures of polymers, having a significant influence in the physicochemical properties of sludge. Intensive researches have also shown that the compositions of EPS could contribute primarily to the structural stability of aerobic granular sludge, which determines the long-term operation effect of aerobic granule system [7]. Moreover, EPS also have a certain contribution for the removal of toxic substance from wastewater because many functional groups such as amine, carboxyl, phosphate and lipid are on the surface of EPS [8]. Sun et al. [9] researched the removal mechanisms of Zn^{2+} and Co^{2+} using EPS from aerobic granules, implying the main chemical groups involved in the interactions between contaminants were apparently alcohol, carboxyl and amino. Thereby, it is desirable to explore the interaction and mechanism between EPS and toxicity substrate, which is beneficial to understand the role of EPS on the production, response and removal of toxicity compounds in practical application of aerobic granular sludge.

Recently, fluorescence spectroscopy, including three-dimensional excitation-emission matrix (3D-EEM) and synchronous fluorescence, has received attention as a potential monitoring tool to characterize the interaction between the toxic compound and EPS [10]. 3D-EEM fluorescence spectroscopy is a rapid, selective and sensitive technique, which could be applied to elucidate the functional groups and element compositions in EPS samples. It could also give the useful information for understanding the dynamics and chemical nature of organic compounds in aqueous media [11]. Compared with 3D-EEM, synchronous fluorescence is one of easy, fast, and non-destructive methods to quantify the binding characteristics of EPS, which could give the information of tyrosine residues or tryptophan residues when $\Delta\lambda$ is stabilized at 15 or 60 nm, respectively [12]. Xu et al. [13] studied the interaction between sulfamethazine (SMZ) and EPS of activated sludge using synchronous fluorescence spectra, indicating that tryptophan residues were involved in the binding between EPS and SMZ.

Based on the above discussion, the objective of this study was to evaluate the potential toxicity of 4-chlorophenol (4-CP) to aerobic granular sludge system and its interaction with EPS in the process of treating nitrogen rich wastewater. 4-CP was selected as a target pollutant because it was widely existed in wastewater as by-products of pulp and paper, dyestuff, pharmaceutical and agrochemical industries [14,15]. A combined use of 3D-EEM fluorescence spectroscopy and synchronous fluorescence spectra was employed to elucidate the interaction between EPS and 4-CP. This research attempts to provide experimental evidence to show the toxicity of 4-CP to aerobic granules system, and it is hopeful for revealing the mechanism between toxic compound and EPS from the point of fluorescence spectroscopy.

2. Materials and methods

2.1. Parent SBR operation

Aerobic granular sludge was cultivated in a lab-scale sequencing batch reactor (SBR) with a working volume of 17 L. The detailed components of high-strength nitrogen wastewater could be found elsewhere [16]. The reactor was sequentially operated with a cycle of 6 h comprising of 5 min for influent filling, 25 min for anoxic process, 300 min for aeration, 2 min for settling, and 28 min for effluent and idle. The volumetric exchange ratio and hydraulic retention time of the reactor were 50% and 12 h, respectively.

2.2. Synthetic wastewater

A synthetic high-strength nitrogen wastewater was used as follows: chemical oxygen demand (COD, as $C_6H_{12}O_6$), 600 mg/L; NH_4^+–N (as NH_4Cl), 200 mg/L; P (as K_2HPO_4) 15 mg/L; $CaCl_2$, 40 mg/L; $MgSO_4 \cdot 2H_2O$, 20 mg/L; $FeSO_4 \cdot 2H_2O$, 20 mg/L and trace element solution 1.0 ml/L according to the previous literature [17]. The influent pH values were adjusted to 7.5 to 7.8 by using $NaHCO_3$ and HCl.

2.3. Experimental design

To evaluate the batch exposure experiments, four column-type reactors were conducted, and each reactor with an internal diameter and a working height of 4 and 50 cm, respectively. Four test concentrations of 4-CP (0, 1, 5, and 10 mg/L) were examined to evaluate the performance of nitrogen removal of aerobic granular sludge systems. Firstly, 200 mL mixture sample (mean size of 1.5 mm) was withdrawn from the parent SBR at the end of aeration. Then, the granular sludge was washed 3 times by using deionized water to remove the external substrate, and resuspended in 100 mL of deionized water. Next, 100 mL of the resuspended sludge and 400 mL of synthetic wastewater were sequentially added into each reactor. Finally, the prepared 4-CP stock solution was added into the batch reactors to achieve the above pre-determined concentrations.

As a result, the initial COD and NH_4^+–N concentrations were about 480 and 160 mg/L in each reactor, respectively. The initial mixed liquor suspended solids (MLSS) concentration and sludge volume index (SVI) were about 5.0 g/L and 20 mL/g, respectively. Afterwards, all reactors were aerated for 6 h with an aeration rate of 1.25 L/min through a fine air diffuser, and DO concentration was controlled above 2 mg/L. The temperature was maintained at the room temperature (25 °C).

2.4. EPS extraction and 4-CP blinding test

The sludge EPS was extracted by using heating extraction method according to the previous research [18]. The polysaccha-